# MORPHOLOGY OF THE EMBRYOS AT GERM DISK STAGE IN *ACHAEARANEA JAPONICA* (THERIDIIDAE) AND *NEOSCONA NAUTICA* (ARANEIDAE)

HIROHUMI SUZUKI AND AKIO KONDO

Suzuki, H. and Kondo, A. 1993 11 11: Morphology of the embryos at germ disk stage in *Achaearanea japonica* (Theridiidae) and *Neoscona nautica* (Araneidae). *Memoirs of the Queensland Museum* 33(2): 645-649. Brisbane. ISSN 0079-8835.

Embryos at the germ disk stage were investigated by electron microscopy in *Achaearanea japonica* (Theridiidae) and *Neoscona nautica* (Araneidae). In both spiders, the germ disk was composed of spherical cells, which had almost no large yolk granules. In the inner part of the embryo, several large yolk granules were packed by cell membrane with various organelles and glycogen granules similarly to lycosid spiders. In *Achaearanea japonica* there were very flat cells which possessed several large yolk granules in the surface region where the germ disk was not formed. In *Neoscona nautica* cells were not observed in that region at all, so the packages of large yolk granules were exposed directly to perivitelline space. The araneid type can be distinguished from the agelenid and theridiid type.

Die Embryonen von *Achaearanea japonica* (Theridiidae) und *Neoscona nautica* (Araneidae) im Stadium der Keimscheibe wurden mittels des Elektronenmikroskops untersucht. Die Keimscheiben der beiden Spinnen bestanden aus sphärischen Zellen, die große Dotterkörnchen nur selten hatten. In dem inneren Teil von dem Embryo wurden manche großen Dotterkörnchen mit verschiedenen Organellen und Glykogenkörnchen von der Zellmembran gepackt, wie im Falle von den lycosiden Spinnen. Im Falle von *Achaearanea japonica* gab es sehr flache Zellen, die manche großen Dotterkörnchen hatten, in dem oberflächlichen Bezirk, wo die Keimscheibe nicht gebildet wurde. Im Falle von *Neoscona nautica* wurden die Zellen in dem Bezirk schließlich nicht beobacht, also waren die Packe von großen Dotterkörnchen direkt in der Perivitellinhöhle entblößt. Der araneide Typus soll sich von dem ageleniden Typus und dem theridiiden Typus unterscheiden. □*Spider, Achaearanea, Neoscona, embryo, germ disk, morphology.*

*Hirohumi Suzuki and Akio Kondo, Department of Biology, Faculty of Science, Toho University, 2-1, Miyama 2 chome, Funabashi-shi, Chiba 274, Japan; 29 October, 1992.*

Three types of germ disk formation are known in the embryos of spiders. In the most common type known in Agelenidae, the germ disk is formed on a hemisphere of the egg as the result of transformation of squamous blastoderm cells in this region into spherical cells. Egg surface of the other hemisphere is covered with squamous cells (Holm, 1952). In the second type known in Theridiidae, the most blastoderm cells converge on the germ disk region and few cells remain on the other hemisphere (Montgomery, 1909). In the third type found in many Araneidae, a rip appears in the blastoderm, so the yolk mass is exposed (Sekiguchi, 1957). Then all blastoderm cells take part in germ disk formation, and any cells are not observed in the region where the germ disk is not formed. A comparative study of the spider embryos at germ disk stage was carried out under light microscope (Kondo and Yamamoto, 1975). The study of germ disk formation under electron microscope was executed in lycosid spiders, whose germ disk formation is the agelenid type (Kondo, 1969, 1970). We had to examine whether remaining cells connect each other or not in theridiid type and whether extreme thin cells exist or not at the superficial region where the germ disk is not formed in araneid type. In present study, electron microscopic investigation of the embryos at germ disk stage was carried out in *Achaearanea japonica* and *Neoscona nautica*.

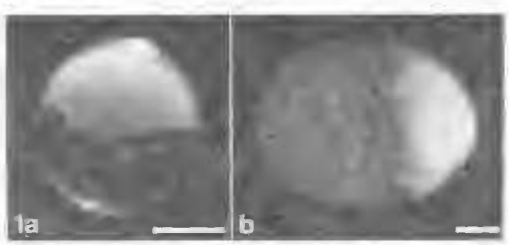
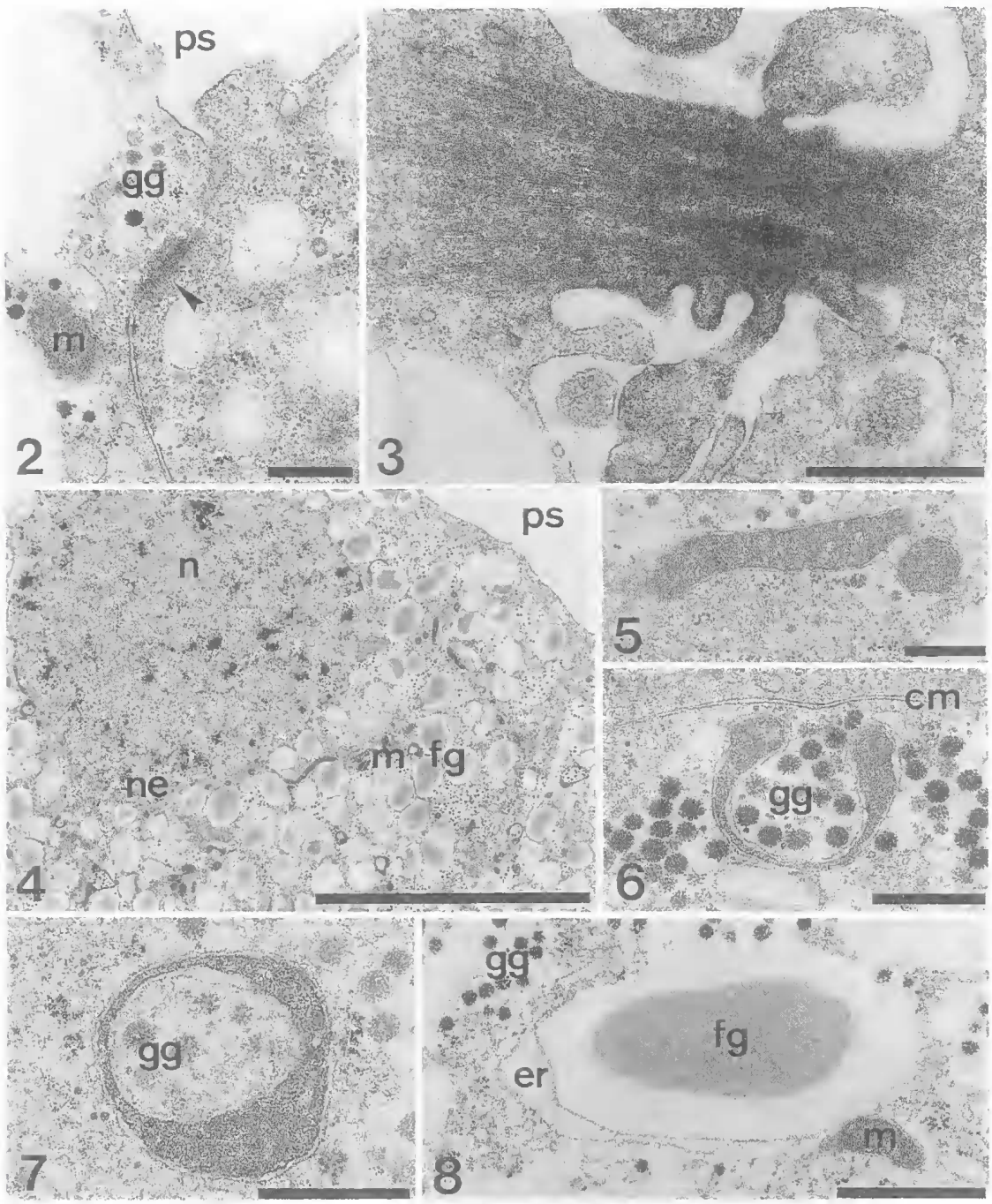



FIG. 1. The embryo at germ disk stage in *Achaearanea japonica* (a) and *Neoscona nautica* (b). In *A. japonica*, a few cells are found on region where germ disk is not formed. In *N. nautica*, exposed yolk mass is found on that region. Scale=0.2mm.




FIGS. 2-8. 2. *A. japonica.* Peripheral region of two germ disk cells. (0.5μm.) Arrowhead: Desmosome-like structure, 3. A mid-body. Many microtubules. (1μm.) 4. A germ disk cell. Main components of cytoplasm are fatty granules (fg) with medium electron dense matrix, and no large yolk granules. (10μm.) n: nucleus, ne: nuclear envelope. 5. Mitochondria have high electron dense matrix and the cristae are found faintly. (0.5μm.) 6. Cup-shaped mitochondrion. (0.5μm). cm: cell membrane. 7. Ring-shaped mitochondrion. (0.5μm.) 8. Fatty granule (fg) lacking complete limiting membrane, but partly enclosed by smooth-surfaced endoplasmic reticulum (er). (1μm.) gg: glycogen granules, m: mitochondria, ps: perivitelline space. Scales in parentheses.

## MATERIALS AND METHODS

*Achaearanea japonica* (Bösenberg and Strand) (Theridiidae) and *Neoscona nautica* (L. Koch) (Araneidae) were used here. In *A. japonica*, the eggs collected in August were used. In *N. nautica*, the eggs laid in glass tubes at laboratory were used. The observation of the live eggs was carried out in liquid paraffin, where the opaque chorion became transparent. The eggs were fixed for 3 hours at 4°C in 2% paraformaldehyde and 2.5% glutaraldehyde solution in 0.1M phosphate buffer, pH 7.4, containing 0.2M sucrose. Through fixation, the eggs were cut in half with a tungsten needle. After rinsing more than one hour with the same buffer containing 0.2M sucrose, the samples were postfixed for one hour at 4°C in 2% osmic acid in 0.1M phosphate buffer, pH 7.4, without sucrose. After rinsing with the same buffer without sucrose, samples were dehydrated in ethanol series, transferred to propylene oxide, and embedded in Quetol-812. Ultrathin sections were cut on a ultra-microtome, LKB-4800, stained with uranyl acetate and lead citrate, and examined under Hitachi HU-12A electron microscope. Thick sections were prepared simultaneously, and stained with methylene blue for light microscopy.

## RESULTS

### *Achaearanea japonica*

The eggs were spherical and 0.5mm in diameter. Typical theridiid type germ disk formation was observed (Fig. 1a). At 25°C, the eggs took 24 hours to the germ disk stage after oviposition.

The germ disk was formed as a single layer composed of spherical cells, but the cells piled up in its central region. The diameter of germ disk cells was about 30μm, and that of nuclei was about 15μm. Desmosome-like structures were observed between germ disk cells at the superficial region (Fig. 2), but interdigitations were not observed. Mid-bodies were observed rarely (Fig. 3). Narrow cytoplasmic bridge connected cells adjacent to each other, and contained many microtubules.

The main components of cytoplasm were fatty granules, 1-3μm in diameter, with a matrix of a medium electron density (Figs 4, 8). The limiting membrane was often obscure. The germ disk cell had almost no large yolk granules. Fine yolk granules, less than 5μm in diameter, were observed.

Mitochondria had a high electron dense matrix, and the cristae were found faintly (Fig. 5). Many figures of mitochondria showed oval or curved bars, and several showed cups (Fig. 6) or rings (Fig. 7).

Smooth-surfaced endoplasmic reticula were often found enclosing fatty granules (Fig. 8). Rough-surfaced endoplasmic reticula were not observed.

Typical Golgi bodies were rare. Vesicles were generally observed in the cytoplasm. The glycogen granules, 0.1μm in diameter, were very high electron dense, and scattered.

The superficial region where the germ disk was not formed was occupied by remaining flat cells. These cells were about 100μm in length, about 25μm in thickness at the central part, but often less than 1μm near the peripheral one (Fig. 9). The diameter of nuclei was about 13μm. Desmosome-like structures were observed between remaining cells (Fig. 9).

Several large yolk granules occurred in these remaining cells. The largest yolk granule was 20μm in diameter. Vesicles were sometimes arranged along the large yolk granules (Fig. 10).

The interior of the embryo was filled with yolk packages composed of several large yolk granules, various organelles and glycogen granules, and enclosed by cell membrane (Fig. 11).

### *Neoscona nautica*

Ellipsoidal eggs of *N. nautica* had longer axis measuring 1.2mm and shorter axis measuring 1mm. At 23°C, 45 hours were needed from oviposition to establish the germ disk (Fig. 1b).

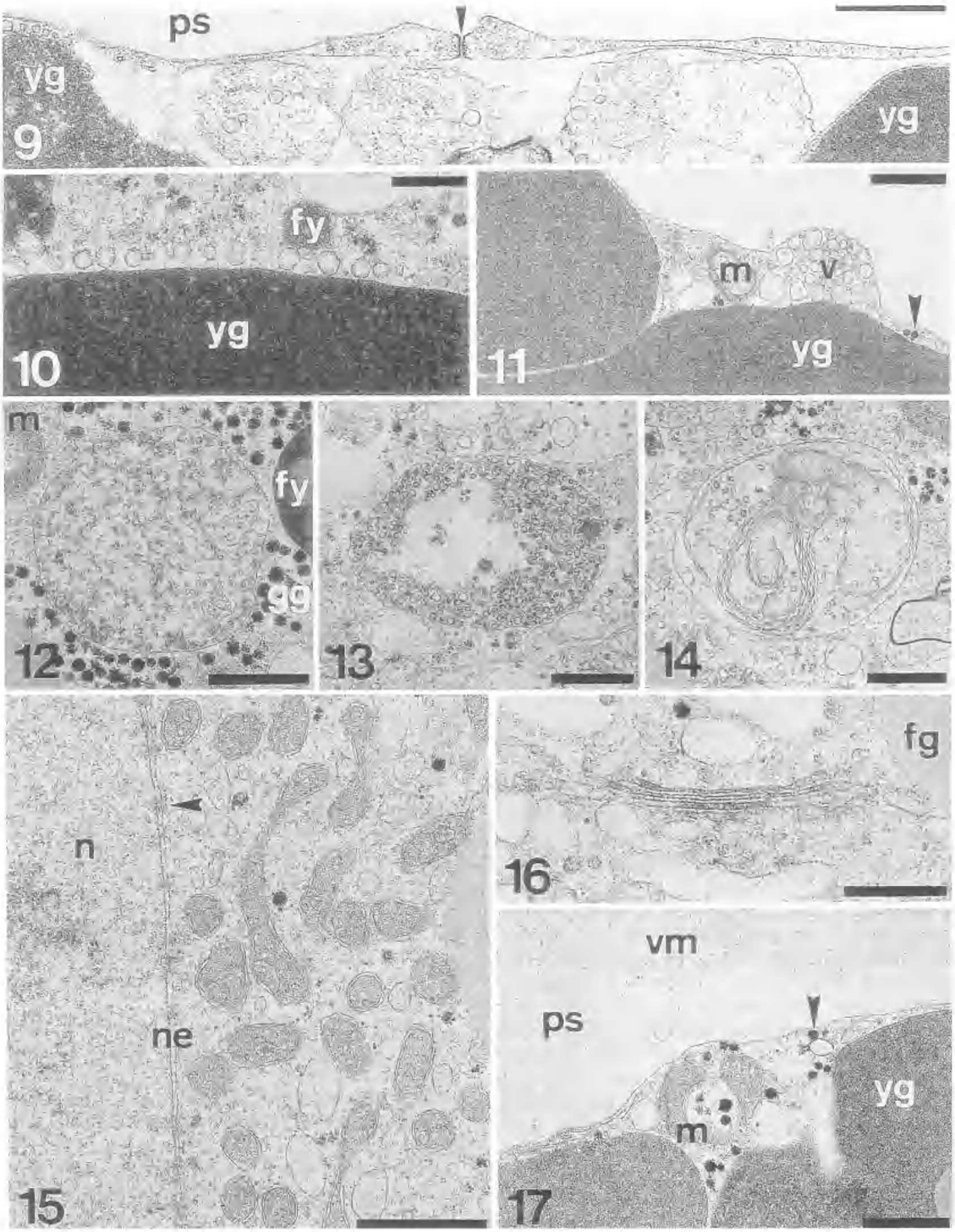
The germ disk was a single layer of spherical cells, but the cells piled up in its central region. The cell diameters were about 45μm, and those of nuclei were about 20μm. Between these cells, there were desmosome-like structures but no interdigitations.

Various types of lysosome-like bodies were observed (Figs 12-14). Mitochondria often crowded around the nucleus (Fig. 15). Several Golgi bodies were observed (Fig. 16). Other components of cytoplasm were similar to those of *A. japonica*

No blastoderm cells were observed in the surface region where the germ disk was not formed (Fig. 17).

## DISCUSSION

In this study, some cup- or ring-shaped




mitochondria were observed. These types of mitochondria were not reported in the embryos of lycosid spiders (Kondo, 1969, 1970), but they were figured in embryo of *A. tepidariorum* (Suzuki and Kondo, 1991). In *N. nautica*, many mitochondria were found surrounding the

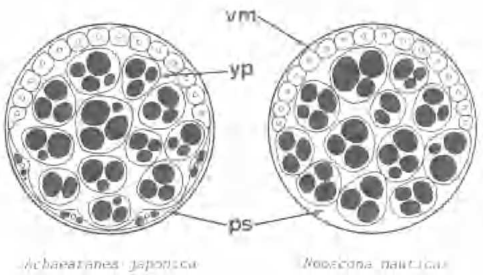



FIG. 18. Schematic figures of embryos at germ disk stage in *A. japonica* (left) and *N. nautica* (right). In both spiders, the germ disk is composed of spherical cells, which have almost no large yolk granules. The interior of the embryos is filled with yolk packages (yp). In *A. japonica*, there are very flat cells which possess several large yolk granules in the surface region where the germ disk is not formed. In *N. nautica*, any cells are not found in that region, so the yolk packages are exposed directly to perivitelline space (ps). vm: vitelline membrane.

nucleus. This phenomenon was reported in lycosid spiders (Kondo, 1969). In *N. nautica*, many lysosome-like bodies, described also in lycosid spiders (Kondo, 1969), were observed, however histochemical studies are needed for final identification.

In both spiders, *A. japonica* and *N. nautica*, interdigitations were not observed, but they were reported in germ disk region of lycosid spiders (Kondo, 1970).

In the inner part of the embryo in both spiders, several large yolk granules were packed by cell membrane with various organelles and glycogen granules. These structures were described as yolk spheres in lycosid spiders (Kondo, 1969). Since nucleus was not observed in them, these packages of large yolk granules were distinguished from yolk cells. In this investigation, detailed observation of yolk cells was not carried out.

The embryo at germ disk stage in *A. japonica* had very flat cells with several large yolk granules (Fig. 18). Distinct differences of cytoplasm were not observed between spherical germ disk cells and flat remaining cells. As in *A. tepidariorum* (Suzuki and Kondo, 1991), except for the large yolk granules and extreme flat shape in the remaining cells, the fine structure of embryo at germ disk stage in *A. japonica* was similar to that in lycosid spiders belonging to agelenid type.

In *N. nautica*, the yolk packages were exposed directly to the perivitelline space (Fig. 18).

## LITERATURE CITED

HOLM, Å. 1952. Experimentelle Untersuchungen über die Entwicklung und Entwicklungsphysiologie des Spinnenembryos. Zoologiska Bidrag fran Uppsala 29: 293-424.

KONDO, A. 1969. The fine structure of the early spider embryo. The Science Reports of the Tokyo Kyoiku Daigaku, Section B 207: 47-67.

1970. Morphological study on the spider's embryonic cells at germ disk stage. Japanese Journal of Developmental Biology 24: 20-21. (in Japanese)

KONDO, A. & YAMAMOTO, N. 1975. Comparative morphology on the early spider embryos. Atypus 63: 13. (in Japanese)

MONTGOMERY, T. H. 1909. The development of *Theridium*, an Aranead, up to the stage of reversion. Journal of Morphology 20: 297-352.

SEKIGUCHI, K. 1957. Reduplication in spider eggs produced by centrifugation. The Science Reports of the Tokyo Kyoiku Daigaku Section B 8: 227-280.

SUZUKI, H. & KONDO, A. 1991. Fine structure of the germ disk in the theridiid spider, *Achaearanea tepidariorum* (C. Koch). Proceedings of Arthropodan Embryological Society of Japan 26: 11-12.

FIG. 9-17. 9-11. *A. japonica* 9. Extreme flat shape in peripheral part of remaining cells. A desmosome-like structure (Arrowhead) is found between cells. (1μm). 10. A large yolk granule included in remaining cell. Arranging vesicles along yolk granule. (0.5μm). 11. Peripheral part of yolk package in inner part of embryo. Large yolk granules, a ring-shaped mitochondrion (m), vesicles (v), and glycogen granules (arrowhead) are packed by cell membrane. (1μm).
12-17. *N. nautica* 12. A lysosome-like body including amorphous matrix. A ring-shaped mitochondrion (m) is found. (1μm). 13. A lysosome-like body including many small vesicles. (1μm). 14. A lysosome-like body including several double membranes or myelin-like structure. (1μm). 15. Mitochondria around nucleus (n) showing nuclear pore (arrowhead) and nuclear envelope (ne)(1μm). 16. A Golgi body. (0.5μm). 17. Superficial region where germ disk is not formed. A yolk package composed of large yolk granules (yg), a cup-shaped mitochondrion (m), glycogen granules (arrowhead), and vesicles are exposed directly to perivitelline space (ps). (1μm). Abbreviations: fg: fatty granule, fy: fine yolk granule, gg: glycogen granules; ps: perivitelline space, yg: yolk granule, vm: vitelline membrane. Scale line in parentheses.